PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 116013/AK/HK	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/IB 2003/004056	International filing date *(day/month/year)* 19.09.2003	Priority date *(day/month/year)* 20.09.2002
International Patent Classification (IPC) or national classification and IPC C12N 15/82, A01H 5/00, G01N 33/53 // C07K 16/08		
Applicant Athena Bioproduction ApS et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 10 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.04.2004	20.12.2004
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Form PCT/IPEA/409 (cover sheet) (January 2004)

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Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

- ☐ international search (under Rules 12.3 and 23.1(b))
- ☐ publication of the international application (under Rule 12.4)
- ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-13 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* 14-16 received by this Authority on 10-09-2004
pages* _____ received by this Authority on _____

☒ the drawings:

pages 1-4 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☐ claims Nos. _____

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 20

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in the Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

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Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-19, 21	YES
	Claims		NO
Inventive step (IS)	Claims	1-19, 21	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-19, 21	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents are considered relevant:

D1) Bouquin T et al, 'Human anti-Rhesus D IgG1 antibody produced in transgenic plants', Transgenic Research, (2002), Vol. 11, No. 2, pp. 115-122
D2) WO0183806 A1
D3) WO0005391 A1
D4) Ma JK et al: 'Assembly of monoclonal antibodies with IgG1 and IgA heavy chain domains in transgenic tobacco plants', Eur J Immunol. vol. 24, no. 1, 1994, pp. 131-138
D5) Li X, Song Y et al: 'A fast neutron deletion mutagenesis-based reverse genetics system for plants', Plant J. volume 27, no. 3, 2001, pages 235-242
D6) US 2002/0123057 A
D7) McCormick Alison A et al, 'Individualized human scFv vaccines produced in plants for the treatment of Non-Hodgkin's Lymphoma: Anti-idiotype responses in vaccinated mice confirm relevance to the tumor Ig', Blood, (2001), Vol. 98, No. 11 Part 1, p. 466a D8) Franconi R et al, 'Functional expression in bacteria and plants of an scFv antibody fragment against tospoviruses', Immunotechnology (Shannon), (1999), Vol. 4, No. 3-4, p. 189-201
D9) McCormick Alison A et al, 'Rapid production of specific vaccines for lymphoma by expression of the tumor-derived single-chain Fv epitopes in tobacco plants', Proceedings of the National Academy of Sciences of the United States of America, (1999), Vol. 96, No. 2, pp. 703-708
D10) Yong-Qiang et al: 'Produce and Characterize Several Classes of Plantibodies (Plant made Monoclonal Antibodies', In

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The use of the expression "C2200-DP-HC-LC" in claim 6 leads to a lack of clarity within the meaning of PCT Art 6. The name of the vector is an arbitrary one, not clearly defined in the description, and therefore it does not seem clear which vector the claim refers to.

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material
      - ☐ a sequence listing
      - ☒ table(s) related to the sequence listing

   b. format of material
      - ☒ in written format
      - ☐ in computer readable form

   c. time of filing/furnishing
      - ☒ contained in the international application as filed
      - ☐ filed together with the international application in computer readable form
      - ☐ furnished subsequently to this Authority for the purposes of search and/or examination
      - ☐ received by this Authority as an amendment* on _____

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

Vitro Cellular and Developmental biology animal, volume 38, abstract 1141, 2002

D1 shows transgenic Arabidopsis plants expressing full-length human IgG1 against the Rhesus D antigen. Binary vectors (C3300-DP-HC-LC) expressing the light and heavy chain from the mas1'2' dual promoter are used. The dual promoter is stated as a fast alternative for simultaneous expression of two transgenes. See page 116 - page 117 left column paragraph 2 and page 118 right column last line-page 119 left column paragraph 1.

D2 shows a method for producing immunoglobulin binding proteins (IgBPs) in plants by transforming cells with a library of at least two different polynucleotides encoding different IgBP polypeptides. An IgBP may comprise a single immunoglobulin chain, multiple identical immunoglobulin chains, or multiple non-identical immunoglobulin chains, or fragments thereof. IgBPs include, e.g., single chain antigen binding proteins, Fabs and Fvs. Plants producing antibody molecules by being transformed with polynucleotides encoding immunoglobulin heavy chains and light chains are contemplated. Mutagenesis can be used to generate a multitude of polynucleotides encoding different variants of native IgBPs, which can then be resolved by expression in an array of eukaryotic cells or plant cells or plants. Functional screens of mutant IgBPs can be made. Assays for screening arrays of IgBPs are shown. See example 1, p. 10 lines 14-22, p. 27 line 27-p. 28 line 9, p. 29 lines 3-15, p. 30 line 23-25, p. 34 line 7-p. 35 line 2, and p. 42 line 7-p. 43 line 4.

D3 shows monoclonal antibodies expressed in plants. Engineered antibodies, made by altering the amino acid sequence of the antibody heavy chain or light chain variable region, are considered. See p. 19 line 32-p. 20 line 9, p.28 lines 12-27, and p. 73 claims 1-5.

D4 shows the expression of the heavy and light chains of a murine monoclonal antibody in Nicotiana tabacum. Production of antibodies is detected using ELISA and to detect the binding function of the assembled antibody. See p. 132 section 2.4.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

D5 shows a screening strategy for identifying a single plant being a deletion mutant in a mixture of plants. The strategy shows how pools of plants are screened and the pools then subdivided into smaller pools. See p. 237, especially Figure 2.

D6 relates to a method of expressing immunoglobulin molecules in eukaryotic cells, a method of producing immunoglobulin heavy and light chain libraries for expression in eukaryotic cells and methods of identifying, selecting or screening immunoglobulins which bind specific antigens. Screening methods in which pools comprising the desired molecules are subjected to an assay in which the desired molecule can be detected are shown. Aliquots of the pools in which the molecule is detected are then divided into successively smaller pools which are likewise assayed, until a pool which is highly enriched from the desired molecule is achieved. Antigen binding in a give pool is detected through an immunoassay. Antibodies against e.g. human immunodeficiency virus antigens, such as envelope antigens, are mentioned. See [0003], [0055] lines 1-4, [0060] lines 13-19, [0119], [0214], [0253] lines 16-25, [0269], [0273] lines 10-14, [0457], and [0458].

D7 is an abstract showing an scFv library cloned into a TMV vector and expressed in plants. The linker between the variable region gene sequences is randomized and small populations of linker variants are screened in infected plants. An individualized vaccine is produced.

D8 shows the expression in plants of an scFv antibody fragment against tospoviruses. The engineered scFv may be valuable for a plantibody-mediated resistance to tospoviruses. See the abstract.

D9 shows the expression of tumour-derived scFvs in tobacco plants. The scFvs are used as vaccines. See abstract and p. 703 right column.

D10 is an abstract discussing the use of plants for producing monoclonal antibodies at low cost. Plantibodies for preventing infective diseases such as HIV and HSV are said to be

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

developed.

The present application relates to a method of producing antibodies in transgenic plants and screening of such antibodies. The invention is stated to provide a solution to the problems associated with prior art techniques of producing antibodies (expensive, contagious antigens, antiglobulin response etc.) by generating human antibody libraries in transgenic plants.

D2 is considered to represent the closest prior art. The difference between the invention according to claim 1 and D1 is that in claim 1 it is stated that sequences contain a multivariable region in which preselected parts which affect the antibodies' ability to bind antigen have been altered.
Claim 1, a product claim, thus contains elements of a process, elements of a product by process claim.
In D1 (see example 1) constructs comprising a heavy and a light chain and promoters for expression in plants are produced. The constructs have altered variable regions. The multivariable region of an immunoglobulin molecule is contained in the variable region. However, since it is stated in claim 1 of the present application that the constructs contain *multivariable regions in which preselected parts of the regions are altered*, the invention according to claim 1 is considered novel in view of the constructs produced in D2. The constructs of claim 1 can be viewed as a selection of constructs produced in D2 (the invention is a selection invention).
By altering preselected parts of the multivariable regions, parts which affect the antibodies' ability to bind to variants of the target antigen, antibodies to antigen variants are produced. By altering the parts of the antibodies that are involved in antigen binding, it seems more likely that new antibodies to antigen variants are produced than if the whole variable region is randomly altered.
It is obvious to the skilled person that antibodies against antigen variants can be made by altering the multivariable region of the molecule. The CDRs of antibodies are commonly altered to generate and identify antibodies with specificity against a desired protein.
However, none of the cited documents refer to alterations to

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

preselected parts of the multivariable regions of the antibodies.
None of the cited document show libraries of antibodies produced in plants, antibodies wherein the regions which affect the antibodies ability to bind to variants of the target antigen are altered. However, such libraries have been produced in other organisms than plants. The skilled person could therefore suspect that production of antibodies wherein the regions which affect the antibodies ability to bind to variants of the target antigen are altered could be made in plants as well. However, it is considered that the expectation of success for combining the teachings of libraries of antibodies in other organisms with the teaching of expression in plants is not reasonable. Consequently, there seems to be no indication that the skilled person would reach a solution falling within the scope of claim 1.
Therefore, the invention according to claim 1 and dependent claims 2-19 and 21 is considered to involve an inventive step.
The invention according to claims 1-19 and 21 is industrially applicable.

Claims

1. A nucleic acid construct comprising:

   (i) a sequence encoding an immunoglobulin heavy chain,

   (ii) a sequence encoding an immunoglobulin light chain, and

   (iii) one or more promoters capable of controlling expression of both sequences in a plant,

   wherein both said sequences contain a multivariable region in which preselected parts of said regions, which affect the encoded antibodies' ability to bind to variants of the target antigen, have been altered by exchanging, inserting or deleting one or more nucleotides as compared to the original said immunoglobulin sequence.

2. The nucleic acid construct as claimed in claim 1, wherein the promoter in (iii) is a dual promoter.

3. The nucleic acid construct as claimed in claim 2, wherein said dual promoter is *mas1'2'* from the *A. tumiefaciens* Ti plasmid.

4. The nucleic acid construct as claimed in any one of claims 1 to 3 further comprising any one or more selected from terminators, enhancers, promotors, or sequences to enable cloning and/or purification of the protein.

5. A vector containing the nucleic acid construct of any one of claims 1 to 4.

6. The vector as claimed in claim 5 wherein said vector is C2200-DP-HC-LC.

7. A plant cell comprising a nucleic acid construct as defined in any one of claims 1 to 4 or a vector as claimed in claim 5 or claim 6.

8. A whole plant, or part thereof comprising a plant cell as defined in claim 7.

9. The seed, and/or propagating material of a plant as claimed in claim 8.

10. A method for the production of populations of antibodies comprising construction of nucleic acid constructs of claims 1 to 4, or vectors of claim 5 or claim 6, and the expression of said nucleic acids or vectors in plants.

11. A method according to claim 10 wherein said plants allows posttranslational modifications and/or overproduction of said immunoglobulin proteins.

12. A method according to claim 10 or claim 11 wherein post-translational modifications of the antibodies are carried out in the plants *in vivo*.

13. A method according to claim 10 or claim 11 wherein post-translational modifications of the antibodies are carried out *in vitro*.

14. A method according to claims 10 to 13 further comprising a method for selecting plants producing antibodies that bind to a specific protein, or fragment thereof, comprising the following steps:

(a) purify recombinant antibodies from a pool of plants expressing said antibodies;
(b) assay said antibodies to determine whether any bind to the specific protein or fragment thereof;
(c) and if the results of step (b) are positive, repeating steps (a) and (b) with the pool of plants sub divided into smaller groups; and
(d) repeating steps (a) to (c) until the plant producing the antibody that binds the specific protein or fragment thereof is identified.

15. The method as claimed in claim 14 wherein the initial pool contains 1000 plants, which is subdivided by a factor of ten in step (c).

16. The method as claimed in claim 14 or claim 15 wherein the assay of step (b) is carried out by means of ELISA.

17. The method as claimed in any one of claims 14 to 16 wherein the specific protein is a viral protein.

18. The method as claimed in claim 17 wherein the protein is an HIV virus protein.

19. The method as claimed in claim 18 wherein said protein is an HIV-1 envelope protein.

20. A pharmaceutical composition comprising an antibody identified by the method as claimed in any one of claims 14 to 19.

21. The use of a nucleic acid molecule as claimed in any on of claims 1 to 4, or a vector as claimed in claim 5 or 6 in the production of a transgenic plant.